The Development of Sarcomas from Transplants of the Hyperplastic Stromal Endothelium of Glioblastoma Multiforme

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EXUBERANT ENDOTHELIAL PROLIFERATION is a common feature of cerebral vessels supplying glioblastoma multiforme. It is present in capillaries throughout the substance of the tumor, but is most pronounced in the abundant network abutting its periphery. The proliferation may be so intense as to fill the vascular lumen and block the circulation of blood; in such instances, it resembles a neoplastic rather than a hyperplastic process. In fact, a number of case reports describing the juxtaposition of glioblastomas and "cerebral sarcomas" are present in the literature, and the accessory tumors have been assumed to originate from stromal vascular cells. However, the frequent association of the sarcomatous growths with the meninges suggests the possibility of such a derivation, with subsequent collision of the growing mass with a coexisting glioblastoma. On the other hand, in two reports the sarcoma had no meningeal connections, and the glioblastoma was associated with intense capillary endothelial proliferation.[1,2]

A considerable number of human glioblastomas have been successfully transferred to the eyes and brains of guinea pigs.[3,4] A similar endothelial proliferation characterizes the vascular supply of the tumors in both transplantation sites, but despite many serial passages, no concomitant sarcomatous tumors have been found. It should be noted, however, that the tissue fragments used for transfer are less than 0.5 mm. in diameter and are derived from the central region of the tumor mass. The vascular stroma of the tumor is relatively scanty in this region, and there is little probability of obtaining sufficient quantities in the inoculum to provide an adequate test of transplantability. The present report is concerned with a series of transplantation experiments utilizing inoculum derived from the periphery of the tumor mass and

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containing both glioblastoma and adjacent tissue with its more abundant component of vascular elements.

Materials and Methods

Signs of increased intracranial pressure become apparent approximately 60 days after brain transfer of glioblastomas and are followed within several hours by death of the guinea pig. Dissection should be carried out immediately; it is relatively simple, for the mass fills half a cerebral hemisphere and is much firmer in consistency than the surrounding brain. For present purposes, a thin investment of cerebral tissue with its component capillary network was left intact, and fragments containing both tumor and vascular supply were used for transfer (Fig. 1). In several instances, the adherent cerebral investment was detached from the tumor and used as the only inoculum.

Anterior chamber transplants obtain their blood supply from the iris and are not attached to the cornea. They are easily removed by fixing the eye with sharp forceps and detaching the cornea by means of a circular incision at the level of the limbus. Fragments of tumor infiltrated with iris pigment constitute the most abundant source of vascular tissue and were used in the present experiments.

Fragments of tissue selected in the manner described were transplanted to the brain of guinea pigs and the subcutaneous space of golden hamsters and BDF_1 mice. The technique utilized in brain transfer has been described in detail elsewhere.[5] Transfer to the subcutaneous space was performed by means of a trocar containing a fragment of tissue measuring approximately 1.0 mm. in diameter.

Guinea pigs, hamsters, and mice were used as recipients in groups of 6. First-generation hamsters and mice received daily intramuscular injections of 4.5 mg. cortisone acetate. Injections were given on the day of transfer and on the 2 succeeding days. Second-generation recipients usually did not require treatment, but occasionally cortisone was repeated at the same dosage. Serial passage of the tumors was carried out in various sites in guinea pigs, standard Dutch rabbits, hamsters, and mice.

Results

The tissue fragments described were transplanted to hamsters, mice, and guinea pigs, and endothelial sarcomas were initiated in all these species.

Initiation in Hamsters

Endothelial sarcomas developed in all hamsters of four of the eight groups bearing subcutaneous transplants of anterior chamber-borne material. The growing tumors reached a palpable size about 2 weeks after transfer, and examination at this time showed neoplastic nodules of endothelial growth adjacent to or contained in surviving glioblastomal tissues (Fig. 2–4). The glioblastomal growths were of short duration and were rapidly replaced by an invasive proliferation of endothelial elements. In the absence of an endothelial component, however, the glioblastomas persisted as solitary tumors. Five such tumors occurred in the 24 hamsters without endothelial growth and, except for a gradual increase in fibrous stroma, their histologic appearance was unchanged over a transplantation period of 3 months (Fig. 5).

Endothelial sarcomas were also initiated in all members of six of eight hamster groups transplanted with brain-grown tissue. The growth rate was more rapid than that of the eye-grown tissue, and the transplants frequently attained a diameter of 1 cm. in 10 days. The increase in size resulted entirely from the proliferation of neoplastic endothelial cells, and no glioblastomal cells were present in the transplants. Occasionally, however, isolated glial growths without endothelial components were found as slowly proliferating tumors (Fig. 6).

In 5 animals the cerebral tissue adjacent to the brain-grown glioblastoma was removed from the tumor by gentle scraping and was used as inoculum. Endothelial sarcomas developed in three of the groups, and there were no instances of glioblastomal growth.

The endothelial tumors derived from eye and brain sources differed histologically in their early growth stages. The endothelium supplied by the eye grew in syncytial-like masses with large nuclei and abundant cytoplasm (Fig. 2 and 4), while that obtained from the brain produced smaller, rounded cells with eccentric nuclei or spindle-shaped cells with fibrils (Fig. 7 and 8). All the cell types were actively mitotic, and the spindle variety were locally invasive from their initiation. Growths of the round-cell type, however, often underwent rapid regression in untreated hamsters, but the continuation of cortisone injection through three serial generations resulted in the production of pale, firmer nodules composed of more anaplastic, polygonal, vasoformative cells and fat spindle-shaped elements bearing fibrils and arranged in fascicular pattern (Fig. 9–12). These cells were locally invasive and possessed the ability to grow in normal, untreated hamsters.

With continued transfer in hamsters, all divergent cell types, including that derived from anterior-chamber tissue, developed this histologic appearance as well as a common growth pattern, and these have persisted through 15 serial passages. The primary transplant grows to a large size, but is susceptible to necrosis and, at death, living tissue is often limited to a thin peripheral layer. Regional node involvement is apparent a month after transfer, and mediastinal and pelvic nodes fill the thorax and abdomen at necropsy. Diffuse dermal metastases occur, but the only observed site of organic metastasis has been the liver (Fig. 18).

Despite the facility of the hamster as a host for alien tissues, it must be noted that it is prone to the production of lymphomas as part of its immunologic reaction to foreign tissue. The lymphomas occur at the site of transfer and replace the transplanted tissue.[6] The endotheliomas under present consideration are guinea pig in identity and may induce a similar reaction. It is essential, accordingly, that such experiments be associated with careful histologic control.

Initiation in Mice

Seven brain-grown glioblastomas were utilized for mouse transfer. There were 6 mice in each of 7 transplantation groups, and endothelial sarcomas developed in all the animals of each of 6 groups.

It should be emphasized immediately that the mouse, like the hamster, occupies a unique position concerning heterologous tissue transplantation. The great majority of alien tumors successfully transferred to its subcutaneous space undergo metaplasia, and the changed morphology is associated with the attainment of the abilities to invade and metastasize in the foreign species. Further, return of the tumor to the species of origin after long serial passage in the mouse is accompanied by reversion of the metaplasia and a re-establishment of the original histologic appearance.[7]

A similar situation prevails in the case of the endothelial sarcoma. In some instances the cells survived without metaplasia, and the absence of the morphologic alteration was not associated with a different behavior. In either case, initiation and growth of the tumor was rapid, and a mass 0.5 cm. in diameter was usually present by the ninth day. Histologic examination at this time demonstrated a metaplastic growth or one closely resembling those found in the hamster (Fig. 13 and 14). The metastatic cells were significantly altered and consisted of spindle-shaped elements with sparse cytoplasm; irregular, bizarre nuclei; and occasional fibrils. Irrespective of morphologic appearances, mitosis was common, and local tissues were invaded.

Death occurred during the fourth week with large ulcerated transplants and extensive metastases in the lung (Fig. 15–17). Invasion of the pancreas was also a common postmortem finding, but appeared to represent an extension of a pre-existing secondary growth in the omentum. Metastases were not found in other organs.

The significance of the metaplastic change is not clear. In other foreign tumors, it appears to be a prerequisite for invasion and metastasis in the mouse, but is not an essential modification in the present instance. Transfer of the same inoculum to mice and hamsters has resulted in metastasizable, metaplastic tumor in the mouse and nonmetastasizable, nonmetaplastic tumor in the hamster. Further, transplantation of the metaplastic tumor from the mouse to the guinea pig's brain or the hamster's subcutaneous space has always been accompanied by a modification in histology and behavior, rendering the cells identical with those of tumors initiated in these species.

Initiation in Guinea Pigs

Endothelial sarcomas developed in animals in seven of the nine groups receiving brain transplants of mixed glioblastomal and vascular tissues, and the remaining groups in this category were limited to growths of glioblastomas. Adjacent vascular tissue, separated from glioblastomas, was used as an inoculum in another experiment. All of the three recipient groups developed endotheliomas.

All but two of the endothelial sarcomas in the mixed-tissue groups occurred as solitary tumors and were not associated with glioblastomal growth. The sarcomas grew rapidly, and the animals rarely survived for more than 3 weeks. At necropsy the growth was soft, highly invasive, and difficult to free from cerebral attachments. Wide areas of brain were replaced, and extension into the opposite hemisphere or through the cerebral thickness was not uncommon. Ventricular invasion was an early occurrence and, even in the case of relatively small growths, the entire system was often filled with the expanding tumor.

The cells varied in structure; some were oval or round with central nuclei and a propensity for vasoformation. Their appearance in brain tissue adjacent to the tumor resembled that of the vascular network characteristic of glioblastomas. Other cells were polygonal in shape with angular boundaries and eccentric nuclei. In less crowded areas, spindle-shaped elements grew in fascicular pattern (Fig. 20–23).

In the two instances of combined glioblastoma and endothelial sarcoma, as well as in the cases of solitary glioblastoma, the growth rates were significantly increased. The usual life span of guinea pigs bearing glioblastomal brain transplants approximates 60 days, yet these animals all bore exceptionally large glial tumors and died at the end of a month. It should be emphasized that the increased growth rates occurred whether or not the tumor contained neoplastic endothelial elements and appeared to relate to some factor differentiating the transplantability of the peripheral glioblastomal tissue from the central portion used in routine transfers. The only histologic alteration in the enlarged growths was a marked expansion in the anuclear areas surrounded by palisading glial cells.

In one instance of concomitant growth, the glioblastoma occupied two-thirds of a cerebral hemisphere, while the endothelial sarcoma was microscopic in size. The latter was situated at the edge of the glioblastoma, but its bulk was largely in the peripheral brain substance and was closely associated with stromal vessels showing pronounced, but not neoplastic, endothelial proliferation. The tumor cells were polygonal in shape, their nuclei were characterized by large central nucleoli, mitosis was common, and adjacent tissue was invaded (Fig. 19). In general, these tumor cells resembled the endothelial cells from an anterior-chamber transplant growing in juxtaposition to a surviving nodule of glioblastoma in a hamster's subcutaneous space.

In a second instance of concomitant growth, both tumors were of equal size, each approximating 0.5 cm. in diameter. They were spherical in shape and separated from each other by a thin layer of nervous tissue. The endothelial sarcoma did not invade the glioblastoma.

The endothelial sarcomas initiated by transplants of vascular tissue alone, without glioblastoma fragments, were identical in behavior and morphologic appearance to the solitary tumors obtained by the transfer of mixed tissues (Fig. 20–23).

Transplantability

Irrespective of origin, the endothelial sarcomas are transplantable in many sites over a wide range of hosts. They grow readily in the brains, eyes, subcutaneous spaces, and intramuscular regions of rabbits, hamsters, and mice; they metastasize in all of these species. They also grow in the guinea pig's eye and muscle, as well as in its brain (Fig. 24). The anterior-chamber growths in this species are rapid, and rupture of the cornea with infection and destruction of the transplant occur before sufficient time has elapsed for metastasis. Subcutaneous and intramuscular transplants are also highly susceptible to infection and to date have not persisted long enough to determine their metastasizing ability.

Discussion

Morphologically, the endothelium in the stromal vessels of glioblastomas in man and in animal transplants is frequently in mitosis, and it is not uncommon to find areas of local proliferation sufficiently intense to obstruct the flow of blood. However, invasion of vascular walls into adjacent tissue with the production of a concomitant tumor is a rare occurrence. In contrast, transplantation of the stromal vasculature of a human glioblastoma, growing in a guinea pig, to other pigs, hamsters, or mice results in endothelial sarcoma development. Accordingly, the acquisition or, at least, the expression of neoplastic properties would appear to bear a relationship to the conditions of transplantation.

Both homologous and heterologous transplantations are concerned in the present experiments. The glioblastoma is a human tumor, but its stroma, like that of all transplanted tumors, is supplied by the new host—in this particular case by the guinea pig. The fact that guinea pig tissue can be successfully transferred to hamsters and mice raises a question of its biologic nature, for only embryonic and neoplastic tissues are capable of heterologous transfer.

The fate of successful embryonic tissue transplants in alien hosts is growth with differentiation, but the fate of the stromal transplants is rapid neoplastic proliferation.[8] Certain tissues, such as Schwann's sheath and pia arachnoid, obtained from adult individuals grow in foreign species at a rate in excess of that found in situ in the primary host, but there is no histologic suggestion of neoplasia. It is conceivable that, in like manner, endothelial cells retain an intermitotic character during the adult life of the host, and that this permits transplantation beyond the capacity of other tissues. However, sarcoma development occurs with such rapidity after transfer, fewer than 6 days in the mouse, as to indicate that neoplasia had been initiated before transplantation.

The major argument that the cells are not neoplastic in the primary host stems from their long residence in guinea pig brains without tumor formation and the extreme rarity of endothelial sarcomas as concomitant growths in man. It should be emphasized, however, that the stromal endothelial cells of a glioblastoma are always intravascular in location, and tumor formation requires the permeation of vessel walls with entrance into interstitial tissues.

The ability to invade vascular walls from a peripheral location and thus gain entrance into the blood stream is not necessarily associated with the ability to permeate vascular walls in a reverse direction and attain an interstitial position. The presence of tumor cells in the blood stream antedates the occurrence of metastasis for long intervals of time both in man and in experimental animals. The fact that the failure to metastasize relates to a failure of circulating tumor cells to reach interstitial tissues, rather than to an inability to grow in the tissues, has been shown by experiment.[9] The organs were removed from a tumor-bearing hamster with demonstrable circulating cells but without metastases, and fragments of the organs were transplanted subcutaneously to normal individuals of the same species. Transfer was followed by a preliminary phase of necrosis affecting vascular walls and an exudation of vascular contents. A new stroma was elicited and, in a short period of time, the histology of the fragment was reconstituted and persisted in intact form. The point of present interest was that nodules of tumor occurred at the sites of vascular exudation.

In brief, circulating tumor cells in the intact host were retained within blood vessels; transplantation of organ fragments resulted in necrosis of vascular walls with discharge of the tumor cells into interstitial tissues, and in tumor formation. It is suggested that the same situation obtained in the case of the endothelial cells in the vasculature of the glioblastomas: they were neoplastic in character, but deficient in the ability to invade vascular walls. It is further suggested that following transplantation the stromal vessels underwent necrosis followed by exposure of the contained endothelial cells to the interstitial tissues of the new host and the production of an endothelial sarcoma. Conceivably, a similar event might follow a surgical procedure or other damage to the abundant vasculature of a human glioblastoma.

The occasional growth of a glioblastoma in the hamster's subcutaneous space, and its persistence far beyond the influence of cortisone, requires some comment. Many previous attempts to transfer the tumor to this site utilizing central fragments of tissue were unsuccessful, and the only variation in technique in the present experiments was the use of peripheral tumor tissue as an inoculum. In any case, growth occurred, and the transplants continued to increase in size throughout the period of observation. The finding applies with significance to the general belief that glioblastomal tissue is incapable of growth in regions other than the brain and the eye and, for this reason, does not metastasize from its primary site in the brain.

The behavior of the glioblastoma in the subcutaneous space is of particular interest in two respects. In the first place, its stroma consists of bands of reticulin-positive connective tissue in contrast to the delicate astrocytic scaffolding of brain growths. The general architecture, however, is identical with that of brain transplants and displays the same resemblance to spongioblastic growths. Further, the subcutaneous glioblastoma is provided with an abundant vascular supply containing the same superfluity of endothelial cells characteristic of brain growths (Fig. 6).

An endothelial hyperplasia is not associated with the growth of transplanted extracranial tumors in the brain. It does occur, however, in man in the vicinity of astrocytomas—a brain tumor generically related to the glioblastoma. There are, thus, two additional sources of rapidly proliferating endothelium for extension of the present study. It is clear that guinea pig endothelium derived from the vasculature of the iris is as susceptible to sarcoma production as is that of the brain. The utilization of astrocytomal stroma will allow expansion of the investigation to another cranial source in man, and the subcutaneous glioblastoma to a subcutaneous source in a different species.

Summary

The transfer to guinea pig brains and to the subcutaneous spaces of hamsters and mice of peripheral fragments of a human glioblastoma multiforme grown in guinea pig brains and containing both brain tumor and adjacent stroma resulted in the formation of endothelial sarcomas. The sarcomas grew progressively in hamsters and mice and metastasized in both foreign species.

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[*Illustrations follow*]

Legends for Figures

Fig. 1. Periphery of glioblastoma in guinea pig brain. Tumor is at left and vascular network at right. × 250.

Fig. 2. Transplant of mixed eye-grown glioblastoma and adjacent stroma, 16 days after subcutaneous transfer to hamster. Endothelial sarcoma is at top and glioblastoma at bottom. They are separated by connective tissue containing iris pigment. × 125.

Fig. 3. This and material in Fig. 4 were adjacent but not connected in the subcutaneous space of a hamster, 10 days after transfer of mixed eye-grown glial and vascular tissue. The tumor is glioblastoma and the pigment is from the iris. × 125.

Fig. 4. Early endothelial sarcoma arising from adjacent glial vasculature. × 250.

Fig. 5. Transplant of glioblastoma from mixed eye-borne tissue, 3 months after transfer, without endothelial growth. Connective tissue separates islands of glioblastoma. × 125.

Fig. 6. Similar transplant of mixed brain-borne tissue 3 months after transfer. Only the glioblastoma has survived. Underlying vascular network is not sarcomatous, but represents a new stroma induced from hamster tissue by the growing tumor. × 250.

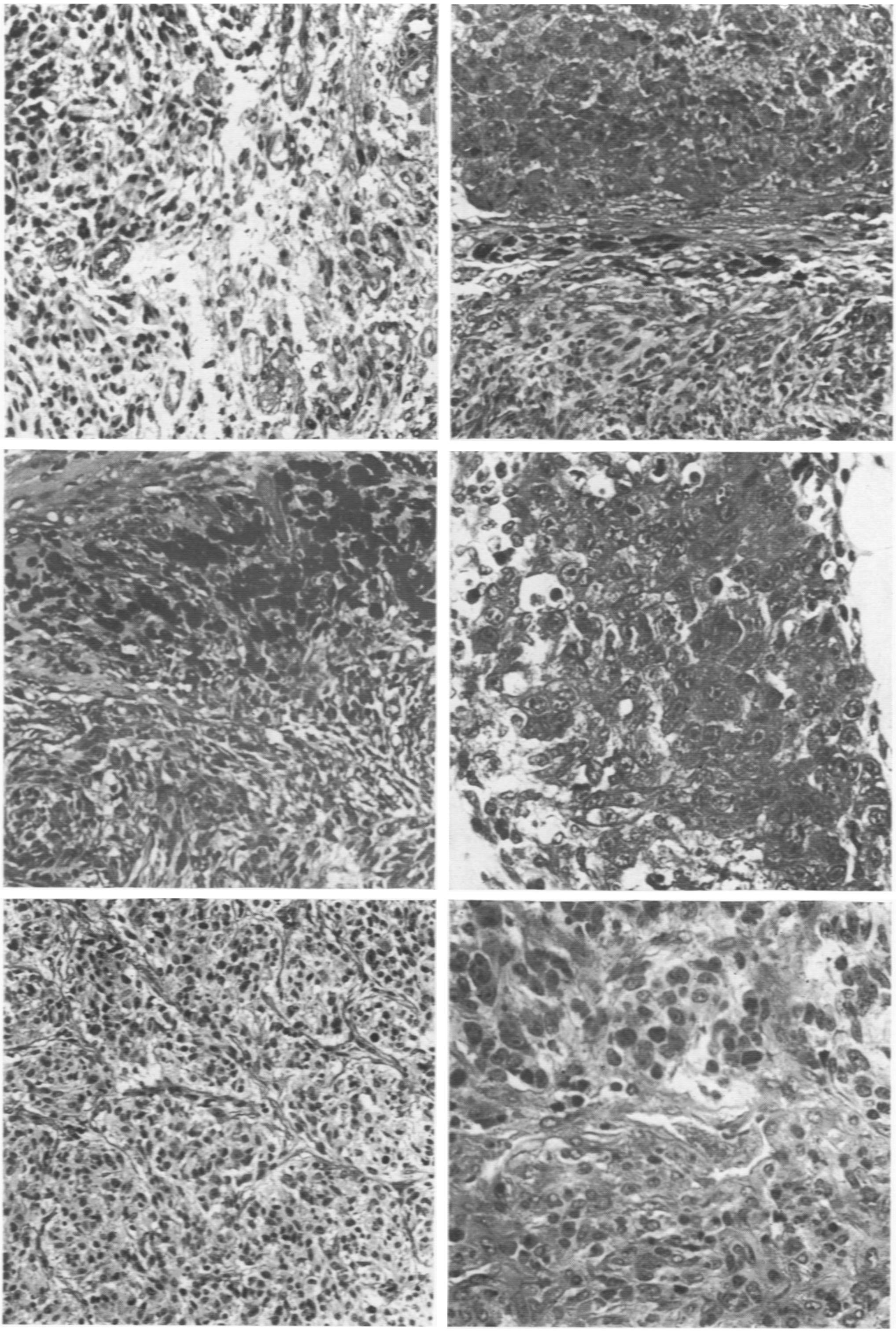

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Fig. 7. Transplant of brain-grown glioblastoma and vascular stroma, 8 days after transfer to hamsters' subcutaneous spaces. Glioblastoma has not survived; tumor is composed of anaplastic polygonal endothelial cells. × 250.

Fig. 8. Transplant of same tissues as in Fig. 7, 10 days after transfer. The cells are less anaplastic, but mitoses are numerous. × 250.

Fig. 9. Transplant of vascular tissue removed from periphery of brain-grown glioblastoma, 14 days after transfer to hamster's subcutaneous space. Contains rounded cells and spindle cells with small fibrils. × 250.

Fig. 10. Third generation transplant of tumor in Fig. 8, 3 weeks after transfer. Contains both rounded and spindle cells. × 250.

Fig. 11. Fourth generation transplant of tumor in Fig. 8, 3 weeks after transfer. Contains both polygonal and fat spindle cells with fibrils. × 250.

Fig. 12. Fifth generation transplant of tumor in Fig. 8, 4 weeks after transfer. Nuclei appear washed out and vary much in size. Cell walls are indistinct. Widespread lymph node metastases. × 250.

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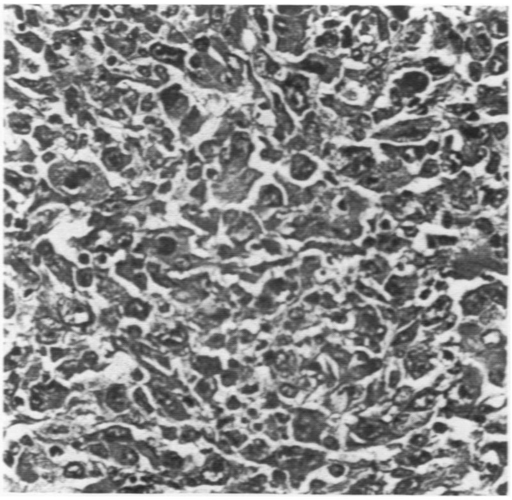

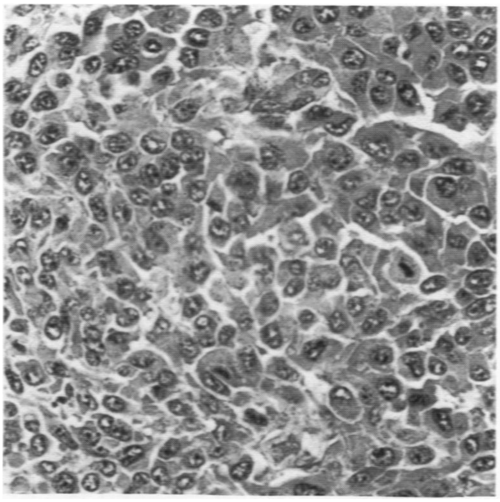
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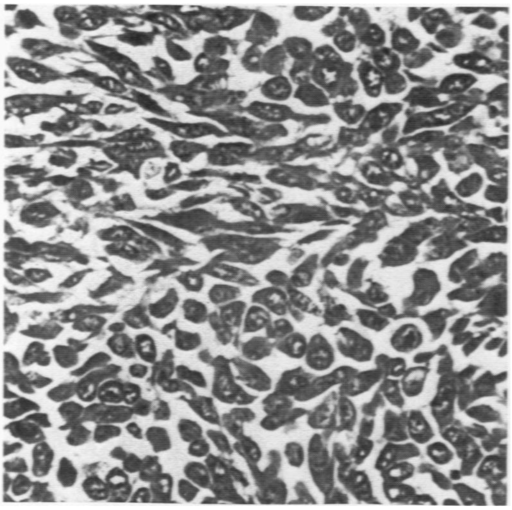

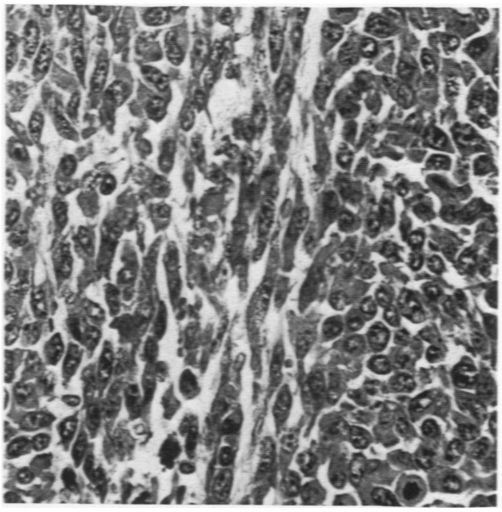
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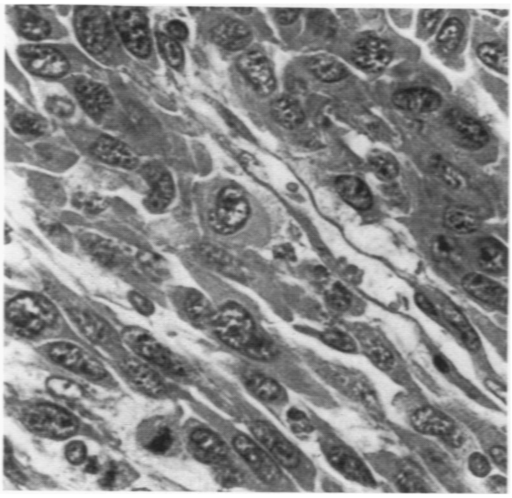

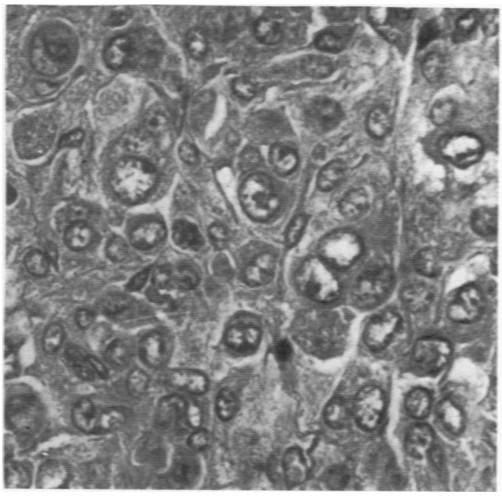
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Fig. 13. Transplant of mixed brain-grown glioblastoma and vasculature, 7 days after transfer to subcutaneous space of BDF_1 mouse. Anaplastic endothelial sarcoma with bizarre cells. × 125.

Fig. 14. Transplant, as above, 10 days after transfer. Cells similar to those found in hamsters' subcutaneous space. × 250.

Fig. 15. Metastasis from BDF_1 subcutaneous space to contralateral axillary node in 18 days. × 250.

Fig. 16. Metastasis to lung on Day 23. × 250.

Fig. 17. Metastasis to omentum with invasion of pancreas on Day 23. × 250.

Fig. 18. Metastasis of mixed brain-grown tissue to hamster's liver on Day 25. × 250.

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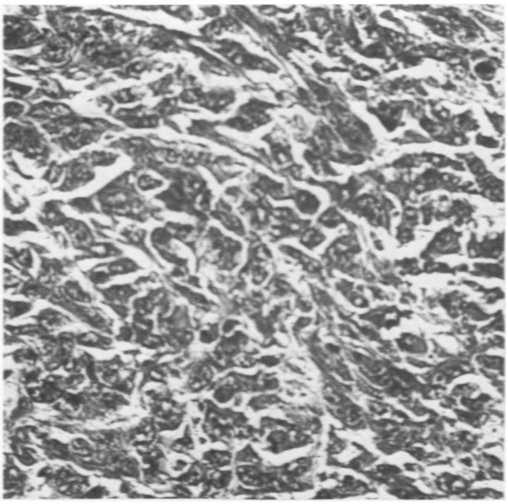

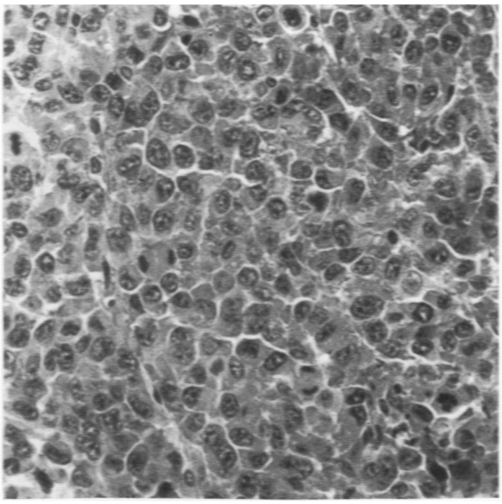

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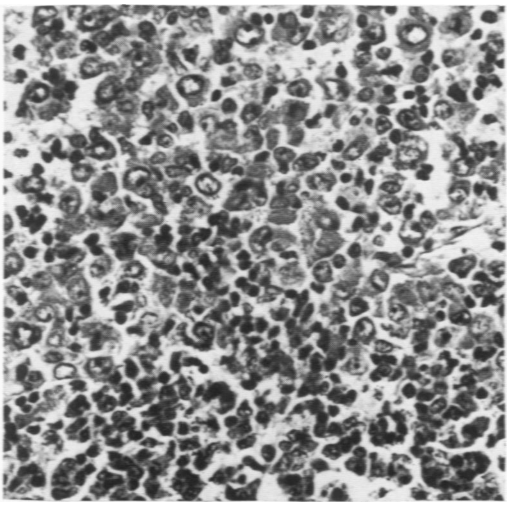

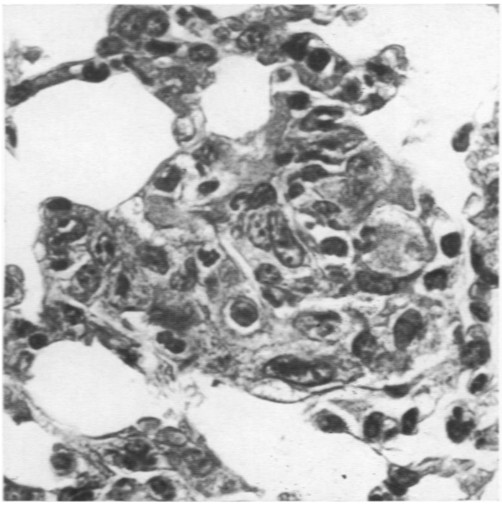

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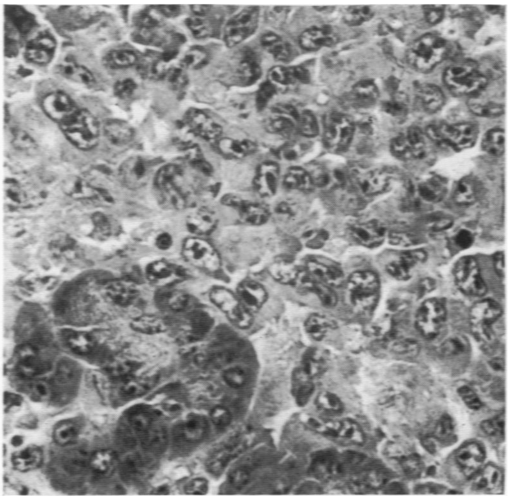

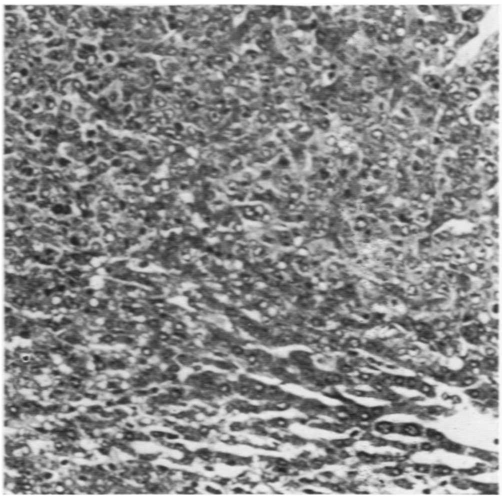

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Fig. 19. Transplant of mixed glioblastoma and vascular tissue to guinea pig's brain, 30 days after transfer. Glioblastoma has grown to fill half a hemisphere; one small nodule of invasive endothelial sarcoma is present in one region of periphery. × 125.

Fig. 20. Transplant of vascular tissue from periphery of guinea pig glioblastoma to guinea pig's brain, 16 days after transfer. Tumor is made up largely of small, spindle-shaped cells. × 250.

Fig. 21. Periphery of large endothelial sarcoma initiated with transplanted vascular tissue in guinea pig's brain. Invasion of Virchow-Robbins spaces is widespread. × 125.

Fig. 22. Diffuse invasion of single cells, as well as invasion of Virchow-Robbins space. The latter surrounds a blood vessel and fills it lumen. × 250.

Fig. 23. Canalized invading columns of endothelial sarcoma cells. × 250.

Fig. 24. Intramuscular transplant of brain-grown endothelial sarcoma in the guinea pig. × 250.

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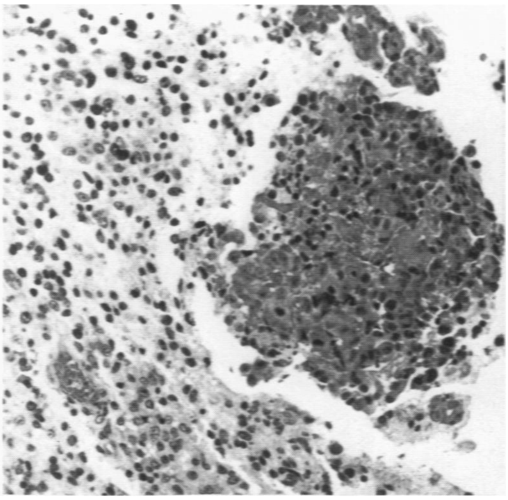
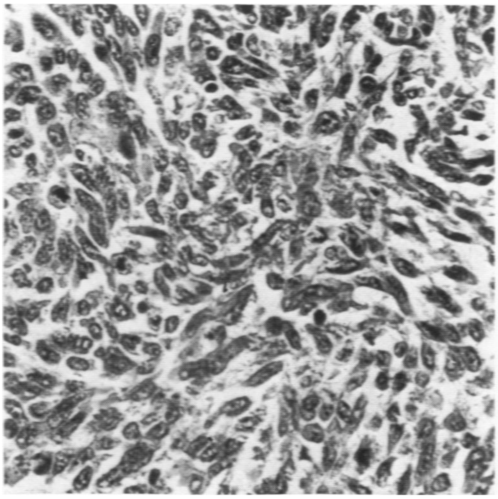
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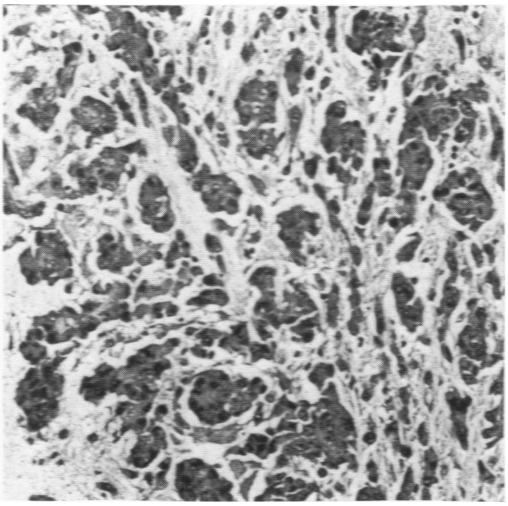
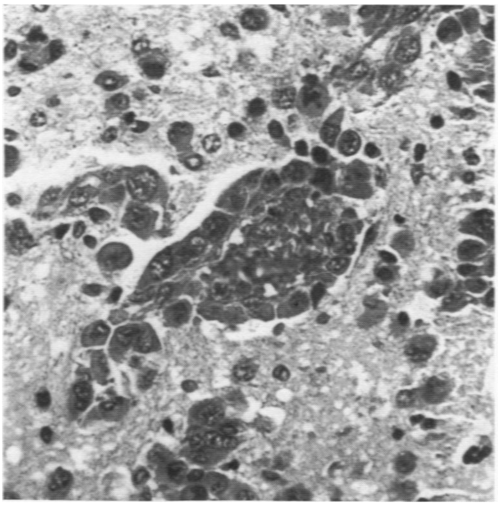
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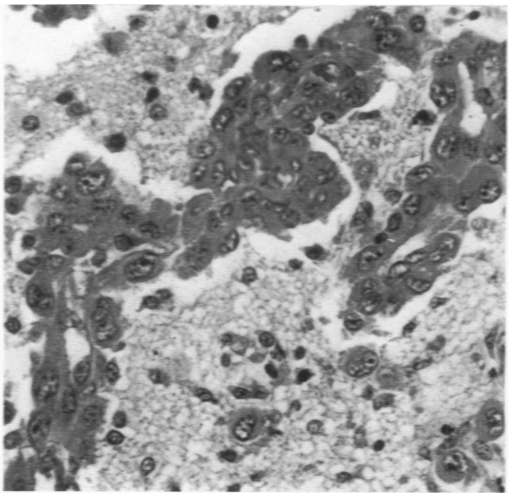
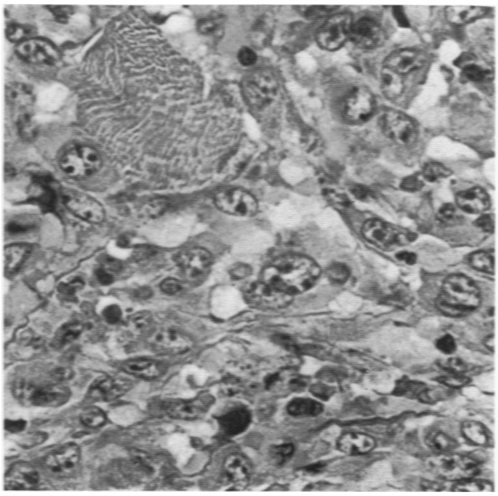
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